Preparation and Purification of Lactulose from Sweet Cheese Whey Ultrafiltrate

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Several methods were developed for efficiently preparing lactulose from the lactose found in cheese whey ultrafiltration permeate. Lactose and organic acid content of this ultrafiltrate were first measured by high-performance liquid chromatography (HPLC). An amount of boric acid that was equimolar to the lactose content was added to the ultrafiltrate, the pH of the solution was adjusted to 11 with either triethylamine or sodium hydroxide, and then the solution was heated (70 °C) for a given interval. Either normal or concentrated whey ultrafiltrate (with lactose concentrations ranging from 4.4 to 17.7 g/100 mL) was used with the resulting yields of lactulose (measured by HPLC) exceeding 80%, based on the starting lactose content. Five different purification procedures were examined for the removal of catalysts, boric acid, and noncarbohydrate whey components from the reaction products. A combination of strong acid, adsorption, and boron selective ion-exchange resins was especially effective for this purpose. The lactulose syrups produced by these methods can be treated by the procedures given, to yield pure crystalline lactulose.

Lactulose (4-*O*-β-D-galactopyranosyl-D-fructose) is a synthetic ketose disaccharide that has several unique pharmaceutical applications (Bircher et al., 1966; Conn, 1978; Méndez and Olano, 1979). This useful sugar was originally synthesized by the isomerization of lactose in basic solution (Montgomery and Hudson, 1930). That process, and others of a similar nature, produces low yields of lactulose, which is difficult and expensive to refine. Most lactulose, therefore, is marketed as an impure syrup that contains varying amounts of related carbohydrates and their rearrangement products (Huhtanen et al., 1980). The high cost and low purity of these preparations have deterred the development of new food applications, even though this sugar has properties (high solubility, nondigestibility, and moderate sweetness) that make it an ideal candidate for certain limited food uses. In an attempt to make lactulose more available for research purposes, we recently (Hicks and Parrish, 1980) prepared pure, crystalline lactulose by isomerizing lactose in the presence of boric acid and base catalysts. This process produces the ketose in high yeilds when pure, refined α-lactose monohydrate is used as a starting material.

Recently, dairy processors have been using a process of ultrafiltration to recover valuable proteins from cheese whey. This ultrafiltration permeate, or ultrafiltrate, may be further refined by ion-exchange treatment and evaporation, to yield pure lactose (Delbeke, 1979). If the lactose in the whey ultrafiltrate could be directly converted to lactulose, however, the energy-intensive lactose refining step could be obviated. This paper, therefore, describes methods for the efficient synthesis and HPLC analysis of lactulose directly from either regular, concentrated, or deionized sweet whey ultrafiltrate. In addition, several methods for the purification and crystallization of these lactulose syrups, including a boron selective chromatographic process, will be described.

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MATERIALS AND METHODS

Preparation and Analysis of Sweet Whey Ultrafiltrate. Fresh sweet whey from full cream cheddar cheese was strained from the vat outlet and fed to a 1200-L storage tank. The whey (at 38 °C) was immediately pumped through a DeLaval Model 340B clarifier to remove fines and a DeLaval Model 340B separator to remove cream, pasteurized at 72 °C for 15 s, and cooled to 46 °C in the regeneration section of a Chester Jensen high-temperature short-time pasteurizer. A sanitized 1200-L storage vat was used as a supply reservoir to feed a home-built Romicon ultrafiltration unit with two Model HF 26.5-45-XM50 membrane cartridges in parallel. The cartridges were operated at an optimum operating temperature of 49 °C and a pressure of less than 25 psi. After filtration of 115 kg of sweet whey 24 h^{-1} $cartridge^{-1}$, the unit was back-blushed, cleaned, and sanitized (200 ppm of sodium hypochlorite). Ultrafiltrate was collected, cooled to 4.5 °C, and then frozen. Prior to reaction, the whey ultrafiltrate was thawed, stirred at room temperature for 1 h, and then filtered through Whatman no. 2 paper. In some cases, the whey ultrafiltrate was then either deionized (Delbeke, 1979) or concentrated, prior to reaction. Total solids and ash values for the whey ultrafiltrates were determined by AOAC (1980) methods 16.032 and 16.035. The composition of a typical unconcentrated whey ultrafiltrate was ash 0.51%, solids 5.8%, and nitrogen 0.051%, pH, 6.3. Ultrafiltrates exhibited minor (ca. 10%) lot to lot variations in these values.

High-Performance Liquid Chromatography. For the determination of lactose and organic acids in whey ultrafiltrate, an HPLC system was used that consisted of a DuPont 870 pump module, a DuPont column compartment (65 °C), a Valco injector (20-μL loop), and a

Gilson Holochrome UV–visible detector (220 nm), to which was attached, in series, a Waters Model 403 differential refractometer and an Omniscribe Model B5217-5 dual pen recorder. The column was a Bio-Rad HPX-87-H^+ (30 cm × 7.8 mm) model eluted with filtered and degassed 0.009 N H_2SO_4 at a flow rate of 0.7 mL/min. Samples of whey (1 mL) were mixed with distilled water (3 mL) and acetonitrile (4 mL). Thse solutions were then shaken and filtered through 0.2-μm nylon 66 filters prior to injection. Lactose quantitation was performed by external standard methodology (Parrish et al., 1980). Organic acids were simultaneously determined (UV detector) according to the method of Marsili et al. (1981).

To determine the sugars present in the lactulose syrups that were produced in this study, the method of Parrish et al. (1980) was used with the following modifications. A DuPont 8800 HPLC controller, a Model 870 pump module, a Valco injector (20-μL loop), a Waters Model 401 differential refractometer, and a Hewlett-Packard Model 3390A integrator were used, in conjunction with an analytical Zorbax-NH_2 column (mobile phase, acetonitrile/H_2O, 80/20; flow rate 2.1 mL/min) or an analytical IBM amino column (mobile phase, acetonitrile/H_2O, 78/22; flow rate 1.75 mL/min). Sample loss during purification and evaporation (prior to HPLC analysis) and variation in injection size were corrected by addition of an internal standard (rhamnose) to the reaction aliquot (see below).

Isomerization of Lactose in Whey Ultrafiltrate to Lactulose. The previous method (Hicks and Parrish, 1980) was modified for the use of whey ultrafiltrate as a starting material. Thus, after determination of the lactose content in filtered regular or deionized (Delbeke, 1979) ultrafiltrate, a equimolar amount of boric acid was added. In a fume hood, triethylamine was then added to the stirred solution until the pH reached 11.0. In a typical experiment, the lactose content was found to be 44.4 mg/mL. To 90 mL of this solution 0.7228 g of boric acid and approximately 4.5 mL of triethylamine were added. The entire mixture was then diluted to 100 mL, warmed to 70 °C in a round-bottomed flask, and then sealed with a rubber septum and reacted at 70 °C in a thermostated water bath. The entire process was conducted in an efficient fume hood. The flask was vented at hourly intervals at which times samples (2 mL) were withdrawn and applied to columns containing 2 mL of Amberlite IR-120H^+, 2 mL of Amberlite XAD-4, and 2 mL of Duolite A-561 (free-base form) (top to bottom order). A 1-mL sample of rhamnose (20 mg) was also applied to the column at that time as an internal standard. The sample was washed through the column with 25 mL of distilled water and the effluent was collected in a 100-mL round-bottom flask, evaporated to dryness, treated with methanol to remove boric acid as previously described (Hicks et al., 1983), redissolved in 2 mL of H_2O and 2 mL of acetonitrile, and then analyzed by HPLC (Zorbax-NH_2 or IBM amino column).

In separate experiments, the reactions were initially titrated to pH 11.0 with 4 N sodium hydroxide solution. It was not necessary to conduct these reactions in the fume hood since no triethylamine was used. Aliquots were removed from these reactions at timed intervals, purified, and analyzed by HPLC.

For the preparation of lactulose from concentrated ultrafiltrate, the following procedures were used. Ultrafiltrate was concentrated at 35 °C under reduced pressure to either 2 or 4 times the original solids concentration. A 90-mL sample of this (2×) concentrated ultrafiltrate, containing 88.4 mg/mL lactose, was mixed with boric acid (1.436 g) and 4 N sodium hydroxide (to pH 11) and then diluted to 100 mL. Another 90-mL concentrated (4×) sample, containing 177 mg/mL lactose, was mixed with boric acid (2.872 g) and 4 N sodium hydroxide (to pH 11) and also diluted to 100 mL. Each reaction mixture was then transferred to 250-mL round-bottomed flasks, sampled, sealed with rubber septa, and then placed in a 70 °C constant-temperature bath. Samples removed at time (30-min) intervals from the more dilute reaction (1-mL aliquots) and the more concentrated reaction (0.5-mL aliquots) were mixed with 1 mL of rhamnose (20 mg/mL) internal standard and applied to columns consisting of (top to bottom order) 3 mL of Amberlite IR-120-H^+, 2.5 mL of Amberlite XAD-4, and 4 mL of Amberlite IRA-743 free-base form. (The IR-743, boron selective resin, purchased in the free-base form was preconditioned to remove all excess hydroxide ion by washing with 50 mL of distilled water/mL of resin.) Samples applied to the columns were followed by a 30-mL distilled water wash. All effluents were collected in 100-mL round-bottomed flasks, evaporated to dryness at reduced pressure (35 °C), redissolved in 4 mL each of H_2O and acetonitrile, and analyzed by HPLC (amino column).

Purification Methods for Crude Lactulose Syrups. A 900-mL sample of ultrafiltrate was analyzed for lactose content, then equimolar boric acid was added, the pH was adjusted to 11.0 with triethylamine, and the entire solution was diluted to 1 L. After 5 h of heating at 70 °C, in a sealed flask that was vented hourly, the resulting amber solution (pH 10.9) was cooled and divided (in a fume hood) into five equal portions. Each portion was processed by a different experimental purification method as described below.

Method 1. The solution was slowly percolated (5 mL/min) through a glass column (3.5-cm diameter) containing (top to bottom) 170 mL of strong acid resin (Amberlite IR-120-H^+), 30 mL of adsorptive resin (Amberlite XAD-4), and 50 mL of weak base resin (Duolite A-561 free base form). The column was washed with 500 mL of additional distilled water and all effluent was collected in a 2-L round-bottomed flask.

Method 2. The solution was treated with the same strong acid and adsorptive resin as Method 1. In this case, a strong base resin (54 mL of Amberlite IRA-400) in the hydroxide form was used. The column wash was 500 mL.

Method 3. The solution was treated with the same resins as method 2 except that the strong base resin was used in the bicarbonate form.

Method 4. The solution was treated with the same quantities of strong acid and adsorptive resins as in Methods 1–3. The bottom resin was a boron selective weak base anion-exchange resin (350 mL of Amberlite IRA-743). The column wash was 1 L.

Method 5. The solution was treated with the same quantities of strong acid and adsorptive resins as in methods 1–3. The bottom resin consisted of a mixture of boron selective resin (190 mL of IRA-743) and bicarbonate form strong base resin (24 mL of IRA-400). The column wash was 1 L.

The resulting effluents from these processes were evaporated to dryness at 35 °C, under reduced pressure. The dry product from process 1 was treated (Hicks et al., 1983) with methanol to remove boric acid. This resulting dry product, and those from the other four processes, was then analyzed to determine the relative effectiveness of each purification method.

Crystallization of Lactulose. Lactulose was crystallized from the isomerization reactions by methods sim-

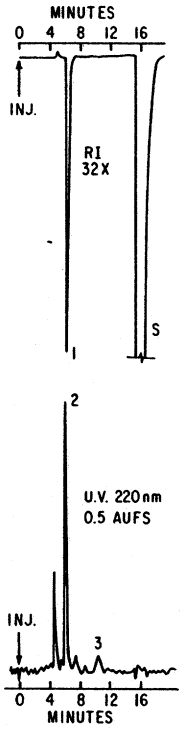

Figure 1. HPLC of whey ultrafiltrate components. Dual detection of lactose (1) by differential refractometer and citric acid (2) and uric acid (3) by UV detection. Bio-Rad HPX-87H$^+$ column. For chromatographic conditions, see Materials and Methods.

ilar to those given previously (Hicks, 1981; Hicks and Parrish, 1980; Pfeffer et al., 1983) with the following modifications. In a typical experiment, 4.8 g of the dried product from method 4 was dissolved in about 10 mL of absolute methanol in a 25-mL round-bottomed flask. This flask was then sealed with a rubber septum, heated at 55–60 °C in a water bath, and kept in a gentle rocking motion. This process was continued for several days, during which crystals eventually began to form. After 5–6 days, the crystallization was judged to be complete and the thick crystalline mass was isolated on a filter and washed with 5 mL of methanol. The product, 1.56 g, had a mp of 157–163 °C. The mother liquors were evaporated to dryness and then treated again with warm methanol (7 mL), as described above, to yield a second crop, 1.27 g, mp 152–159 °C. A third crop was similarly obtained, 0.3 g, mp 157–162 °C. No more crystals were isolated from a fourth crystallization attempt. These three crystalline fractions were combined, and a portion (2.3 g) was recrystallized to yield two crops of crystals. In order to recrystallize the lactulose, crystals were first dissolved in a minimal amount of water and then evaporated to dryness. The dry residue was dissolved in 4.6 mL of methanol and heated at 55–60 °C, as previously described. Recrystallization was faster and more efficient than initial crystallization, taking only 4 h. The isolated crystals (first crop), 1.50 g, had mp 162.5–165.5 °C. Authentic lactulose (Aldrich) had mp 162.5–167 °C. Mixed mp was 161–166 °C. Elemental anal. Calcd for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48. Found: C, 42.19; H, 6.28. Boron: <3 ppm. Second crop: 0.55 g; mp 155–160 °C.

RESULTS AND DISCUSSION

Analysis of Whey Ultrafiltrate. The lactose and organic acid content of whey ultrafiltrate may be conveniently and accurately determined by HPLC. The HPX-87H$^+$ column, designed for organic acid analysis, may also be used, as shown here, for the determination of

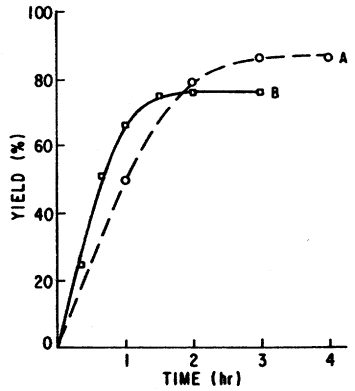

Figure 2. Yield of lactulose prepared from whey ultrafiltrate. (A) pH adjusted with triethylamine; (B) pH adjusted with dilute sodium hydroxide.

lactose in whey ultrafiltrate (Figure 1). The top chromatogram (RI detection; 32×) shows only lactose (peak 1, 6.3 min) and acetonitrile (peak S), whereas the bottom chromatogram (UV detector, 220 nm, 0.5 AUFS) records citric acid (peak 2, 6.2 min), uric acid (peak 3, 10.4 min), and other minor and normal whey components. The organic acid profile is of value in determining whether a whey ultrafiltrate sample is suitable for lactulose production. A highly fermented whey ultrafiltrate would contain a diminished amount of lactose and increased levels of lactic, acetic, butyric, and other organic acids that may interfere with the isomerization reaction and subsequent purification. In our studies, the unconcentrated ultrafiltrates used contained between 44 and 53 mg/mL lactose and no detectable levels of fermentation-derived organic acids.

The precision of this method was studied by analyzing, in duplicate, five samples of the same whey ultrafiltrate solution. The mean value for lactose concentration was 52.52 mg/mL. The standard deviation between duplicates was 0.61 mg/mL with a coefficient of variation of 1.16%. The standard deviation between samples was 0.88 mg/mL with a coefficient of variation of 1.67%.

To determine the accuracy of the HPLC method for whey lactose, a standard amount of lactose was added to whey ultrafiltrate samples. The lactose concentration before and after the standard addition was determined on six separate samples. The amount of lactose found, after addition, was 100 ± 2.8% (SD) of the expected (theoretical) value.

It is noteworthy that citric acid and lactose nearly coelute in this chromatographic system. The response of citric acid in the differential refractometer at this concentration is negligible, however, relative to the response of lactose. Likewise, lactose has negligible absorbance at 220 nm and, therefore, does not affect the quantitation of citric acid at that wavelength.

Preparation of Lactulose from Whey Ultrafiltrate. The lactose in the whey ultrafiltrate was found to be readily convertible to lactulose, in high yields. The reactions were monitored by HPLC (amino column), and the yields of lactulose at various times are shown in Figure 2. When triethylamine as used as the base (curve A), higher yields of lactulose resulted (86% at maximum) but at a slower reaction rate than that seen for the sodium hydroxide catalyzed reaction (curve B). When whey ultrafiltrate that had been deionized and decolorized to remove all whey components except lactose (Delbeke, 1979) was used as a starting material (Figure 3), there was little difference in reaction yields from that of the normal ultrafiltrate reaction. Hence, the presence of normal whey ultrafiltrate salts, peptides, amino acids, and other organic

Table I. Effect of Purification Method on Purity and Yield of Lactulose Prepared from Whey Ultrafiltrate

purification method[a]	overall lactulose yield, %[b]	sugar composition of product, %[b]				carbohydrate lost[b] during processing, % of total	N, %[c]	B, ppm[c]
		tagatose	galactose	lactose	lactulose			
1	75	1.6	6.3	5.8	86.6	13	0.20	<10
2	51	1.4	4.7	5.6	88.3	41	0.21	34
3	76	1.6	5.3	6.7	86.4	11	0.15	10 700
4	67	2.0	10.0	9.0	79.0	15	0.04	37
5	77	1.8	6.8	5.9	85.5	10	0.04	43

[a] Purification methods all involve treatment of product with cation-exchange resin (H^+ form) and adsorptive resin. Additional specific treatments include the following: method 1, weak base resin (free-base form) and methanol treatment; method 2, strong base resin (OH^- form); method 3, strong base resin (HCO_3^- form); method 4, boron selective resin; method 5, boron selective and strong base resin (HCO_3^- form). For additional details, see Materials and Methods. [b] Determined by HPLC. [c] Determined by a commercial laboratory.

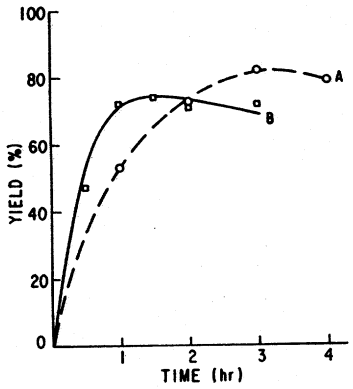

Figure 3. Yield of lactulose prepared from deionized whey ultrafiltrate. (A) pH adjusted with triethylamine; (B) pH adjusted with dilute sodium hydroxide.

compounds do not adversely affect the conversion of lactose into lactulose.

Purification of Whey Ultrafiltrate Derived Lactulose Syrups. The lactulose prepared in the reactions described in Figures 2 and 3 was purified by using the previously published method (Hicks et al., 1983) of removing cations with IR-120-H^+ resin, decolorizing with XAD-4, removing strong acids with A-561, and removing boric acid by repeated treatment with methanol. While this process effectively removes most ionic and noncarbohydrate species, it was observed here, and previously (Hicks et al., 1983), that failure to exert great care when following the methanol treatment leads to the production of unknown side products and a resultant decrease in product yield.

Because the workup of the syrups appears to affect product yield and quality nearly as much as the reaction conditions themselves, we tested several alternative methods for sample purification. A larger scale isomerization reaction (900 mL of ultrafiltrate) was performed and the reaction solution was divided into five equal parts. Each portion was treated by one of five different methods described earlier (see Materials and Methods). The final products from each of these treatments were examined by HPLC for sugar composition and also analyzed for boron and nitrogen content. Table I describes the results of these experiments. Method 1 is the previously developed method for removal of ionic material. It consists of treating the syrup with strong acid resin, adsorptive resin, and weak base resin and, finally, methanol treatment to remove boric acid. This allows a high overalll yield of lactulose and an extremely low level of boron residue. Unfortunately methanol is present in these products in high concentrations. Removal of this methanol is incomplete, even after several hydration, dehydration cycles. In addition, almost 11% of this product consists of unidentified side-reaction products.

Treatment of the reaction solution with a strong acid resin (hydrogen form), adsorption resin, and a strong base resin (hydroxide form) by method 2 effectively removes boric acid but, as is well-known, irreversibly binds reducing sugar was well. In this case, over 40% of the total starting carbohydrate was lost by this mechanism.

Using the strong base type resin, but in a bicarbonate form (method 3), resulted in less adsorption of sugar. This resin, however, was not effective in removing boric acid from solutions. Strong base ion-exchange resins (hydroxide form), therefore, remove sugar, as well as boric acid. Weak base ion-exchange resins or bicarbonate form strong base resins do not absorb either effectively.

The use of a relatively new ion-exchange resin that is selective for boron compounds, in conjunction with strong acid and adsorption resins (method 4), however, produced a lactulose syrup that contained very little residual boron, extremely low levels of nitrogen-containg compounds, and a very low level of unidentified compounds (less than 6%). A relatively large amount of the reaction product (15%) was, however, adsorbed by the resin. This adsorption was found later to be due to the resin being partially in the hydroxide form. When this resin is carefully washed to remove excess hydroxide ion (see following section), adsorption of sugar is prevented.

One may substitute the previously described bicarbonate form resin for a portion of the boron specific resin (IRA-743) if desired (method 5). In this case, the purity of the lactulose syrup was the highest acheived. Boron and nitrogen residues were very low and the final syrup was crystal clear in color. Adsorption of sugar was almost negligible, so that the overall yield of lactulose was nearly 80%.

It is noteworthy that the boric acid levels in the method 5 purification procedure were essentially the same as those in method 4, even though half the amount of boron selective resin was used. This suggests that even smaller amounts of this resin (IRA-743) may be required, in an efficiently packed column. This resin is unusual in that it is a weak base ion-exchange resin that is capable of complexing boric acid. It is not simply the amine functionality, however, that binds boric acid, but rather an *N*-methylglucamine residue that accomplishes this function. The open-chain D-gluco configuration of this group is an exceptionally strong complexation agent for boric acid, forming either bi- or tridentate borate ester complexes. Our further studies on the use of this resin for the removal of borates from sugar solution suggest that its ionic form has a strong effect on the capacity and efficiency of the resin, and its use in the free-base or bicarbonate form is strongly recommended. A detailed description of these

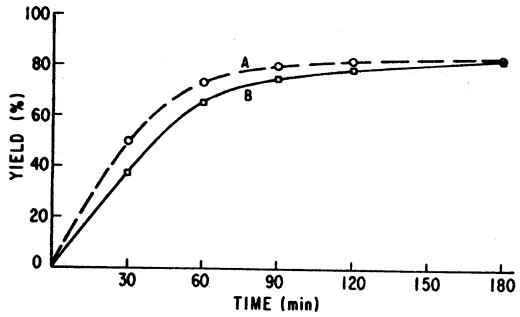

Figure 4. Yield of lactulose prepared from concentrated whey ultrafiltrates. The lactose content for the reaction samples was (A) 80 and (B) 160 mg/mL.

effects will be published elsewhere.

Preparation of Lactulose from Concentrated Whey Ultrafiltrate. Transportation of whey to processing facilities is often preceded by concentration to a high solid level. The use of concentrated whey ultrafiltrate as a starting material for lactulose synthesis was therefore studied here. Whey ultrafiltrate was concentrated to either 2 or 4 times the original concentration of solids. The lactose concentrations of these samples were 8.8 and 17.7 g/100 mL, respectively. The yields of lactulose from these two reactions (Figure 4) were nearly identical after 3 h of reaction. The more concentrated reaction appeared to react at a somewhat slower rate but ultimately reached the same value as the less concentrated reaction. These reactions were analyzed by HPLC (IBM amino column) after aliquots were purified by method 4 (see Materials and Methods), in which IRA-743 resin in the free-base form (carefully washed to remove all hydroxide form) was used.

Figure 5 shows a chromatographic analysis of a reaction sample (3 h) of the 2-fold concentrated ultrafiltrate. With the purification procedure used here, the final lactulose syrup contained the following relative percentages of sugars: tagatose 1.0%, galactose 4.1%, lactulose 86.8%, and lactose 8.1%, with no unidentified peaks. Only 4% of the total carbohydrate was adsorbed to the ion-exchange resins in this case, producing an overall yield for lactulose of 83%. Purification of these lactulose syrups (prepared from both concentrated and unconcentrated ultrafiltrate) by this modified method 4 procedure is therefore recommended.

Crystallization of Lactulose. Lactulose may be crystallized from the purified products of these reactions. Initial crystallizations of lactulose from reaction products are often slow and low yielding and may require several weeks to complete. This is due, in part, to the effect of side products that are formed during the isomerization reaction and those produced during the traditional methanol treatment for removing boric acid. By using method 4 or 5, as described here, reaction side products are effectively removed. Because boric acid is also adsorbed by the boron selective resin, methanol treatment is eliminated, as are the associated byproducts (Hicks et al., 1983). Syrups produced by this process are pure enough for most purposes without subsequent crystallization. When crystalline lactulose is desired, however, these syrups are ideal, high-yielding sources.

CONCLUSIONS

Lactose in whey ultrafiltrates may be efficiently converted into lactulose. The presence of normal whey ultrafiltrate components does not interfere with the boric acid assisted, isomerization reaction. Concentrated whey ultrafiltrates also serve as ideal starting materials for these

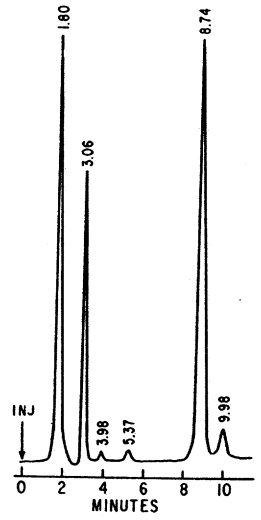

Figure 5. High-performance liquid chromatographic analysis of lactulose syrup. IBM amino column. Chromatographic conditions are given under Materials and Methods. Peak identities: 1.80 (minutes), solvent peak; 3.06, rhamnose (internal standard); 3.98, tagatose; 5.37, galactose; 8.74, lactulose; 9.98, lactose.

reactions. The reaction products resulting from these isomerization methods may be purified to remove boric acid, base catalysts, and nonsugar components by using special ion-exchange techniques. A combination of strong acid, adsorption, and boron selective type resins is especially effective for this purification. Lactulose may be crystallized from those purified syrups to give a pure, nonhygroscopic product. HPLC is a valuable tool for the analysis of normal whey ultrafiltrate components, as well as for determining the composition of isomerized lactose syrups produced from them.

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